SYNTHESIS AND CHARACTERIZATION OF NOVEL METAL ORGANIC FRAMEWORKS FOR BIOLOGICAL APPLICATIONS

A Synopsis

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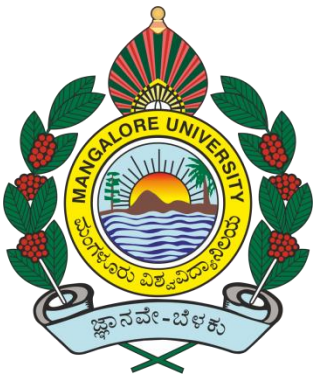

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Synthesis and Characterization of Novel Metal Organic Frameworks for Biological Applications

Introduction

On the therapeutic front, antibiotics have widely been used in human medicine and as prophylaxis or growth promoters in the food animal industry. The level and complexity of resistance mechanisms exhibited by bacterial pathogens have increased with indiscriminate therapeutic and prophylactic use of antimicrobial agents. Recently, WHO announced antimicrobial resistance (AMR) as one of the major threats to humankind and the environment. It is reported that AMR is responsible to cause mortality among 7,00,000 individuals every year around the globe [1]. It has also been estimated that the death toll by way of AMR would increase to 10 million by the year 2050, which would further decrease the gross domestic product (GDP) by 3.50 per cent, resulting in an overall global economic loss to the tune of USD 100 trillion [2].

Of late, the focus has primarily been shifted towards alternative therapeutic strategies to combat AMR apart from the routinely employed antibiotics. The alternative therapeutic strategies devised include phytobotanicals and phytochemicals, synthetic peptides, probiotics, polyclonal antibodies and nanoparticles [3][4]. Recently, nanoparticles (NPs) have emerged as a novel alternative approach to overcome bacterial drug resistance encountered globally. The potential anti-bacterial activity of NPs is mainly due to their large surface area to volume ratio than their bulk material which allows the binding of a large number of ligands on the surface of NPs and hence its complexation with receptors present on the bacterial cell surface [5][6]. Metal–organic frameworks (MOFs) are among the most interesting hybrid porous materials which have been considerably progressed in recent years [7]. These unique complex and porous chemical compounds are essentially forming with a building-block

approach by connecting metal ions with organic ligands which act as linkers between diverse metal centers [8][9]. The high internal surface area and versatile architectures of MOFs make them promising candidates for development of advanced drug delivery systems, nitric oxide storage, imaging and sensing applications [7].

Metal Organic Framework

Metal group of materials constructed from metal ions/clusters -organic frameworks (MOFs) are a revolutionary and organic ligands that link together via self-assembly, richness of both organic and inorganic chemistries [10]. These materials are constructed by joining metal-containing units [secondary building units (SBUs)] with organic linkers, using strong bonds (reticular synthesis) to create open crystalline frameworks with permanent porosity[11]. Although MOFs offer seemingly limitless possibilities for self-assembly and consequently, nearly infinite structural diversity, the methods for their synthesis remain ripe for optimization and expansion. Various approaches have been developed for the synthesis of MOFs with desired structural (topology, pore size, and shape) or functional properties[11]. The chemistry of MOFs has provided an extensive class of crystalline materials with high stability, tunable metrics, organic functionality, and porosity.

Development of research in the area of metal organic framework is relatively novel and excellent example for interdisciplinary research. The term MOFs, was introduced in 1995 [12] and now widely accepted. Because these structures were constructed from long organic linkers, they encompassed void space and therefore were viewed to have the potential to be permanently porous[13].[14] proved that permanent porosity of metal organic framework was obtained by measurement of nitrogen and carbon dioxide isotherms on layered zinc terephthalate MOF. To prepare MOFs with even higher surface area (ultra high porosity) requires an increase in storage space per weight of the material. Longer organic linkers

provide larger storage space and a greater number of adsorption sites within a given material. [15].

Many of these materials are designed to use either as drug carriers for therapeutic applications. An alternative approach is to deliver the drug with a chaperone that encapsulates the drug. This is a ideal strategy that can be an effective means of improving both the pharmacokinetic and pharmacodynamic properties of the parent drug[16]. These large systems are particularly interesting for the delivery of anticancer agents as they clinical applications,

Non-Typhodal Salmonella

Salmonellosis is an important food-borne disease caused by *Salmonella enterica*, a Gram-negative intracellular facultative pathogenic bacterium that comprises more than 2600 serovars [17]. It is estimated that *Salmonella* causes about 1.35 million infections, 26,500 hospitalizations, and 420 deaths in the United States every year (CDC, 2020). Among the enteric bacterial pathogens, 99 per cent of the animal and human infections are caused by *Salmonella enterica* serovars and produce about 94 million infections and 155,000 deaths worldwide every year [18]. Among non-typhoidal *Salmonella* (NTS) serotypes, *S.* Typhimurium and *S.* Enteritidis are commonly associated with human infections in North America, Latin America, Europe, Asia, and Africa.

Entero Aggregative *Escherichia coli*

Enteroaggregative Escherichia coli (EAEC) is one of the six diarrheagenic *E. coli* pathotypes, which is defined as *E. coli* isolates that produce the aggregative adherence pattern (AA) on cultured epithelial cells (HeLa and/or HEp-2) *in vitro*, The AA pattern is characterized by a bacterial arrangement that resembles stacked bricks on both epithelial cells and the coverslip EAEC is a cause of diarrheal disease in children and adults, living in both

developing and developed countries, are associated with cases of persistent diarrhea and diarrheal outbreaks worldwide and are distributed across the four main *E. coli* phylogroups (A, B1, B2 and D) Moreover, high rates of resistance to ampicillin and trimethoprim-sulphamethoxazole, but not to quinolones, have been observed. The pathogenicity of EAEC is characterized by the colonization of the intestinal epithelium, followed by the production and secretion of enterotoxins and cytotoxins, which result in secretory diarrhea

Methicillin-Resistant *Staphylococcus aureus*

The increasing prevalence, perseverance, and adaptability of bacterial resistance to antibiotics is a vexing healthcare problem; one which results in high morbidity/mortality and healthcare costs exceeding $20 billion annually.1–5 Awide range of infectious strains now exhibit antibiotic resistance. Common examples include MRSA (methicillin-resistant Staphylococcus aureus), Pseudomonas aeruginosa lung- and wound-infections, VREs (vancomycin- resistant Enterococci), bacterial pneumonia strains, and urinary tract-infections (UTIs), as well as a host of infections that occur in association with human conditions such as AIDs, and intestinal/colon breaches.6–8 The frequency of community- acquired methicillin-resistant Staphylococcus aureus (MRSA) increased more than seven-fold from 1999 to 2006.

Objectives of the present investigation are:

1. Synthesis and characterization of metal and organic nanoparticles
2. To Synthesize and design of novel metal organic framework (MOFs) and their systematic characterization
3. To evaluate the biocompatibility of these novel metal organic frameworks through various biological assays

The thesis has been organized into six chapters:

Chapter 1 Introduction

This chapter deals with the general introduction and scope of the present investigation. The background of infectious diseases, their treatment strategies, and the significance of MOF-based drug formulations with a focus on antimicrobial therapy have been described initially. This introduction also provides brief background information on MOF and its advantages in the field of biomedical applications.

Chapter 2 Literature Survey

In this chapter, review of literature initially deals with literature related to antimicrobial resistance, and its global status and indian status, reviewed by WHO surveillance reports as well as impact estimated by US Centers for Disease Control and Prevention (CDC), National Center for Disease Control (NCDC), India surveillance reports on multidrug-resistant organisms (MROs). The most prominent bacterial infections associated with diarrhoea worldwide are Escherichia coli (*E. coli*). Based on the host clinical symptoms and type of virulence factor, in this study selected Enteroaggregative *E. coli* (EAEC), non-typhoidal salmonella (NTS) can cause bacteremia, gastroenteritis, and subsequent localised infections. Salmonella's serotypes including S. Typhi and S. Paratyphi, which typically cause severe acute typhoid syndrome (enteric fever) in humans, are host-specific, and methicillin-resistant *Staphylococcus aureus* (MRSA) also demonstrates resistance to a number of other antimicrobial substances, including aminoglycosides, macrolids, lincosamides, tetracyclines, and fluoroquinone.. One efficient technique for addressing environmental problems, such as water treatment, is photocatalysis. Further significance of photocatalytic disinfection of MOF was explained. Moreover, Structure and nomenclature of MOF, different types of nanosized MOF synthesis and applications in different fields such as catalyst, Separation and gas storage, drug delivery, imaging, computer

tomography, positron emission tomography, photodynamic therapy, photothermal therapy and MOF based antimicrobial agents also mentioned. Further the toxicity of MOF and importance of essential oil citral was described in this chapter, Although it is important to evaluate the safety of these novel materials for public health and the environment, the toxicity of MOF is currently insufficiently defined. It is one of the key concerns that need to be taken into account in order to fulfil their potential applications. Organic linker toxicity, metal ion toxicity, fabrication solvent toxicity, and MOF crystal structure are the four key contributors to MOF toxicity. Common solvents used for the synthesis of MOF include water, diethylene formamide (DEF), dimethyl formamide (DMF), and acetone

Chapter 3 Materials and Methods

This chapter explain the experiments conducted to gather requisite data to fulfill the objectives of this study. Initially materials used in this study such as chemicals, bacterial isolates, software, oligonucleotide, antibiotic disc, essential oil, instrumentations and *Galleria mellonella* larvae collection and propagation was described. Further the methods of validation of MDR-typical EAEC, NTS and MRSA isolates, antibiotic susceptibility testing, synthesis and characterisation of ZIF-8(Fe), synthesis and characterisation of ZIF-8-citral, *in silico* studies like ADMET and molecular docking methodology was described. *In vitro* drug loading and drug releasing studies was conducted followed by antibacterial efficacy was assessed (MIC and MBC) with dose- and time-dependent in vitro growth kinetics of MDR-EAEC treated with MOF. Further, *in vitro* membrane permeability assay and Photocatalytic activity and photocatalytic disinfection property, *In vitro* antioxidant assays (DPPH, ABTS and reducing power assay) antibiofilm assay (Congo red binding assay, Inhibition of biofilm forming ability of MDR-EAEC, NTS and MRSA by Crystal violet staining), Haemolytic Assay, Effect of MOF on commensal gut flora, Propagation and maintenance of *G. mellonella*, survival study, melanisation assay and statistical analysis was described

Chapter 4 Results and Discussion

This chapter deals with the results and discussion of the investigation.

Validation of MDR-typical EAEC, NTS and MRSA Isolates and Antibiotic Susceptibility Testing

The revived cultures of EAEC, NTS and MRSA isolates were re-validated by employing a standardized PCR assay targeting genus-specific *invA* gene for Salmonella spp., and serovar-specific *sdf* gene for S. Enteritidis. Based on the antibiotic susceptibility test results, three MDR-EAEC, NTS and MRSA strains were selected to study antimicrobial efficacy of the synthesized MOF by both in vitro and in vivo experiments: The MDR pattern of the confirmed NTS, EAEC and MRSA isolates was evaluated by antibiotic susceptibility testing using disc diffusion assay.

Synthesis of CuS NPs, ZIF-8(Fe), ZIF-8(Fe)-Citral and Characterisation

In this study, CuS NPs, ZIF-8(Fe) nanoparticles were synthesized using an aqueous solvent under vigorous stirring. The surface plasmon resonance (SPR) of synthesized ZIF-8(Fe) nanoparticles showed an intense peak at 215 nm. Initially, the modified ZIF-8 with dual metal clusters (zinc and iron) and 2- methyl imidazole employing organic linkers were synthesized. It was designed to deploy ZIF-8(Fe) as a drug delivery vehicle after the complete characterizations. This essential oil was encapsulated with pre-synthesized ZIF-8(Fe). The development of highly crystalline ZIF-8(Fe) nanoparticles was also evidenced by powder diffraction data. Furthermore, the Debye-Scherrer equation revealed that the average crystal size was 36.10 nm.

In silico studies

The ADME analysis (physicochemical properties, water solubility, lipophilicity, pharmacokinetics, drug likeness and medicinal chemistry) of the selected phytochemicals tested (citral) was carried out by Swiss ADME software. Essential oil citral tested revealed drug likeness with no violation to Lipinski's rule of five. To assess possible essential oil binding interactions, molecular docking was attempted on the structures of dispersin from EAEC (PDB ID: 2JVU) and outer membrane protein ompC from NTS (PDB ID: 3UU2) using the constructed ligand structure.

MOF Based Drug Loading and Drug Release Kinetics

Citral was found to be loaded with ZIF-8 based on UV-DRS and FTIR spectroscopy analyses. The DLE of citral was found to be 19.3%, while the DLC was determined to be 77.2 % after 24 h of incubation. This confirms conclusively how effectively citral interacts with ZIF-8(Fe).

Antibacterial Studies of MOF (MIC, MBC, Tolerance, Time Kill Studies and Membrane Permeability Assay)

The MIC and MBC of ZIF-8(Fe) encapsulated citral was determined as a measure of their *in vitro* antimicrobial activity. The MIC and MBC values of ZIF-8 laden citral against the MDR strains of EAEC and NTS were found to be 62.50 µg/mL and 125 µg/mL, respectively. In order to evaluate the membrane permeability and thereby the antibacterial mechanism of the synthesized ZIF-8(Fe), ONPG- based assay was performed. In the present study. A concentration-dependent increase in the permeability of both *E. coli* and *S. aureus* was observed while treating the bacterial cells with ZIF-8(Fe) at MIC and MBC levels.

Photocatalytic Studies (Dye Degradation and MDR Disinfection)

Methylene blue (MB) and rhodamine b (RhB) were selected as model cationic dyes to explore the photocatalytic activity of ZIF-8(Fe). In order to evaluate the photocatalytic potential, one isolates of MDR- EAEC, NTS and MRSA (taken as representative bacterial strain) test strains were treated with MIC and MBC levels of ZIF-8(Fe) under irradiation at controlled incubation.

Antioxidant Studies (DPPH, ABTS and Reducing Power Assay)

The antioxidant scavenging activity of the synthesized ZIF-8(Fe) was assessed by DPPH, reducing power and ABTS assays. Later, a reducing power assay of the was performed.

Hemolytic and Gut Flora Inhibition Studies

In this study, the possible hemolytic capability of synthesized ZIF-8(Fe) was assessed in chicken red blood cells. The adverse effect of ZIF-8(Fe) on the commensal gut lactobacilli were investigated by the microbroth dilution method employing *L. acidophilus* MTCC 10307 and *L. plantarum* MTCC 5690.

Antibiofilm Assay (Congo Red Assay, Crystal Violet Assay)

The results of Congo Red binding assay to determine the slime production as an indication for the biofilm forming ability employed for the two MDR-EAEC,NTS and MRSA strains is presented. The biofilm forming ability of MDR–EAEC, NTS and MRSA isolates at different times i.e., 24 h and 48 h was assessed by 24 -well microtiter plate assay using crystal violet staining and the absorbance was read at OD_{595} nm.

In vivo Survival Studies

The *in vivo G. mellonella* experiments for evaluation of toxicity of MOF against the increasing dose of MOF were performed as per the procedure The survival curves plotted for the evaluation of toxicity of MOF was using the Kaplan-Meier method is presented Melanin production, as a marker of immune response was investigated in all the larvae groups

Chapter 5 Summary and Conclusion

This chapter summarises and concludes up the findings conducted for the present work. The candidates successfully synthesized a dual metal cluster MOF by using (Zn & Fe) and 2-methyl imidazole as a organic linkers with sodalite morphology. Formation of highly crystalline MOF was characterised and subjected to various biological assays. In this study reports synthesized ZIF-8(Fe) can be employed as an ideal candidates for the application of photocatalytic disinfection as well as drug delivery vehicle due to its high catalytic efficiency, drug loading and drug releasing properties.

Chapter 6 Future Perspectives

This chapter deals with future perspectives and challenges of MOF biological applications. MOF are crystalline materials with a high porosity that can be used for a variety of biological applications. In this study successfully evaluate the catalytic and drug delivery potentials of synthesized materials along with preliminary toxicity studies. Even though the extensive study require with varying catalytic media conditions, in vivo ADMET studies and changing active drug formulations, these are experiments have been left for the future.

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